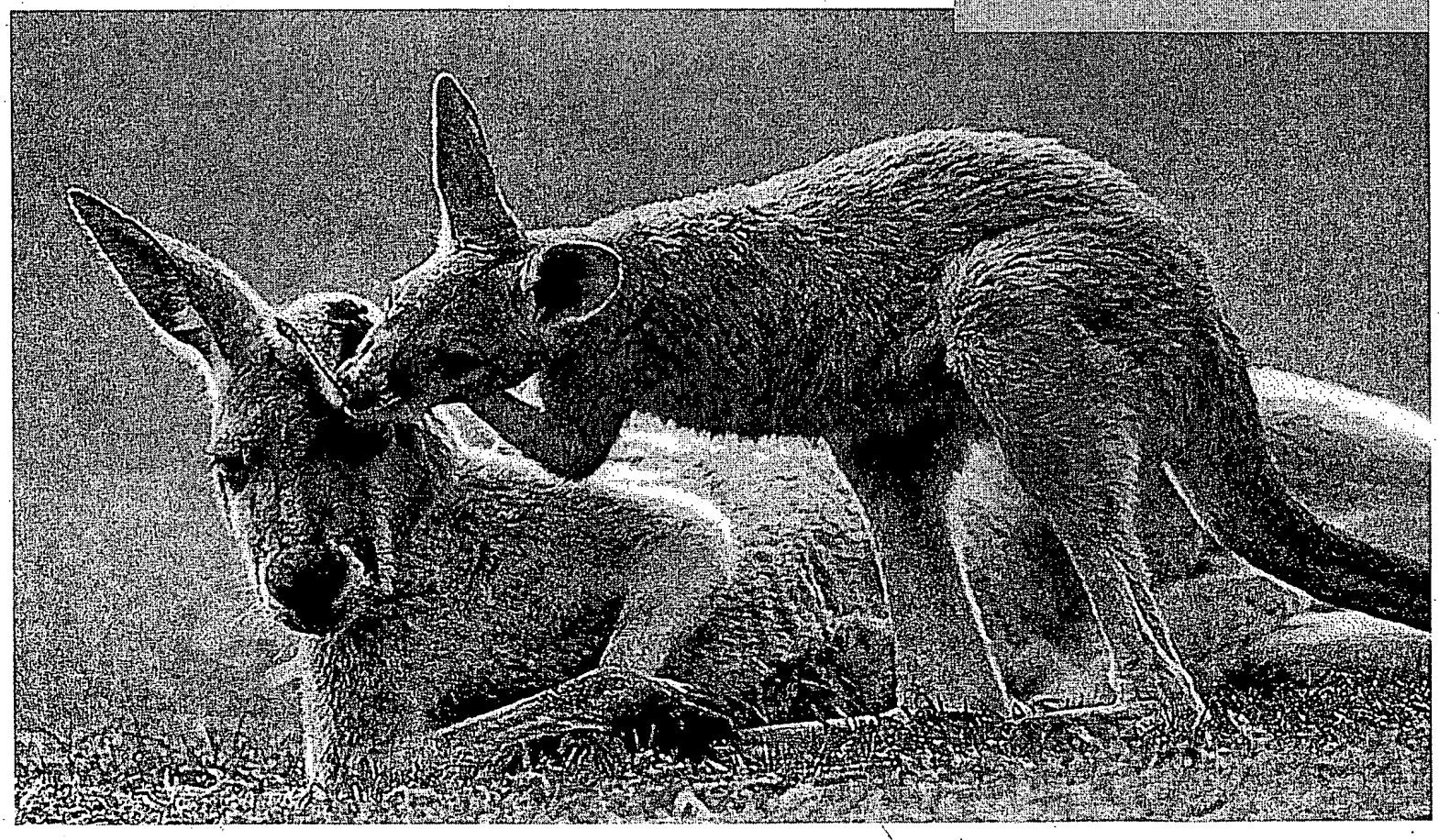
Roche

Roche Molecular Biochemicals

The Complete Guide for Protease Inhibition

featuring the c∅mplete Protease Inhibitor Cocktail Tablets

Convenience and Reliability



In just minutes, serine proteases can destroy the proteins you have spent days isolating. In the past, PMSF and DFP were used to eliminate this problem. However, they provided uncertain protection for protein samples due to their poor stability and solubility in aqueous solutions. Now, protecting your proteins has a simple solution .... an aqueous solution, made with Pefabloc SC.

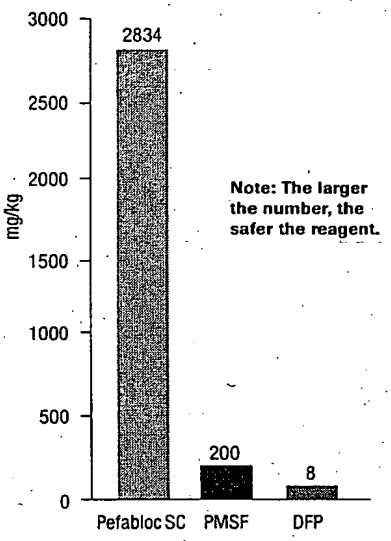


Figure 1. Safety of Pefabloc SC compared to PMSF and DFP. Mice were given oral doses of each inhibitor, and the LD_{50} in mg/kg was determined.

Convenience and Reliability with *Pefabloc SC*

Despite the popularity of PMSF and DFP, both have serious disadvantages. Today, Pefabloc SC is the preferred serine protease inhibitor, providing superior protection with unmatched CONVENIENCE and RELIABILITY.

Convenience:

- **Easier to use –** Pefabloc SC is readily soluble in water, and may be added directly to aqueous buffers. Unlike Pefabloc SC, PMSF and DFP are poorly soluble in water. Because of this, stock solutions must be prepared in organic solvents, and only then added to aqueous solutions.
- **Safer to use –** PMSF is a neurotoxin, and DFP is a deadly cholinesterase inhibitor. In contrast, non-toxic Pefabloc SC provides complete protease inhibition without risk to you, or those around you (Figure 1).

Reliability:

- **Improved stability –** Pefabloc SC remains highly active in aqueous solutions, protecting your proteins long after PMSF and DFP have failed. Protease inhibition is sustained even at pH levels above 7.0 and temperatures above 4°C (Figure 2).
- **Maximize inhibition –** Superior solubility and stability in aqueous buffers mean that Pefabloc SC eliminates the guesswork and promotes success! The poor solubility and stability of PMSF make it difficult to maintain an effective concentration, and leaves you questioning whether levels of active inhibitor are high enough to assure total protection.

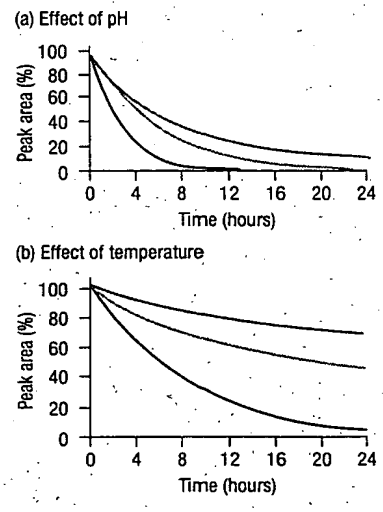


Figure 2. The effect of temperature and pH on the stability of Pefabloc SC. (Ref. 32)
Graph (a) shows the relative stability of Pefabloc SC (5.0 mg/ml) in an aqueous phosphate buffer at 37°C.
- pH 6.5
- pH 7.0
- pH 7.5

Graph (b) shows the relative stability of Pefabloc SC (5.0 mg/ml) in an aqueous phosphate buffer at pH 7.0.
- 4°C
- 22°C
- 37°C

Pefabloc SC: Increase flexibility with a broad range of applications

Pefabloc SC can be used in all applications where the general inhibition of serine proteases is desired. With its high stability and irreversible inhibition mechanism, protein solutions are protected throughout total procedures, such as:

– extraction processes (from animal tissues or cells, plants, bacteria, yeast, and fungi)

– subsequent purification steps

– sample storage conditions

– downstream protein analysis

– biochemical studies where proteins are required.

Pefabloc SC is especially useful to inactivate proteinase K, for example, during pulse field gel electrophoresis (PFGE). With this technique, isolating the genomic DNA requires proteinase K to degrade cellular components, and this highly resilient protease is difficult to inactivate. Pefabloc SC inhibits proteinase K, and protects the stability of restriction enzymes used for further DNA analysis.

Additional Convenience and Reliability with *Pefabloc SC PLUS*

Recent findings indicate that sulfonyl-type serine protease inhibitors like Pefabloc SC and PMSF can bind covalently to proteins. This can occur when the inhibitors are used in high concentrations, or during extended incubation times under alkaline conditions (Figure 3). This interaction adversely affects the tyrosine and lysine residues of a protein, as well as the free amino terminus. The Pefabloc SC PLUS set combines the protease inhibitor Pefabloc SC with a uniquely formulated Pefabloc SC protector (PSC protector). In addition to the benefits already described for Pefabloc SC, it offers *additional* CONVENIENCE and RELIABILITY.

Additional Convenience:

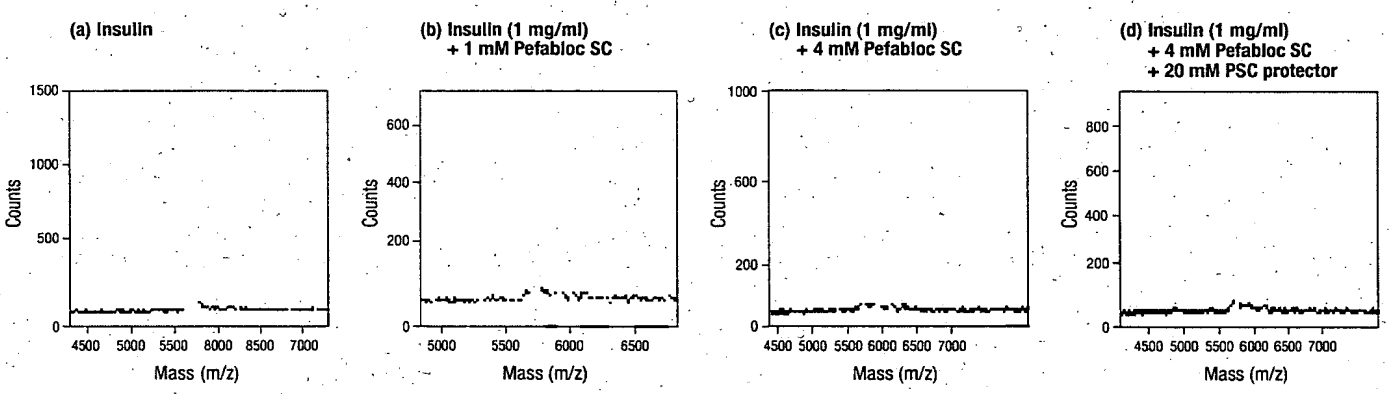
- Simplified, two-reagent system with balanced quantities of reagents.
- Both Pefabloc SC and the PSC protector are stable and non-toxic.

Additional Reliability:

- No covalent binding between proteins and Pefabloc SC, even at high concentrations, extended incubation times, and at alkaline pH (Figure 3).
- No influence on the inhibitory effectiveness of Pefabloc SC (Figure 4).

Additionally available: When it is not possible to replace Pefabloc SC for PMSF in your protocols, try PMSF PLUS, with its own special reagent – the PMSF protector*. As when using Pefabloc SC PLUS, the PMSF protector prevents covalent protease inhibitor-protein binding while having no influence on the inhibitory effectiveness of PMSF (data analogous to results shown in Figures 3 and 4).

Figure 3 (a-d): Mass spectrograms showing the covalent interaction between insulin and the protease inhibitor Pefabloc SC. Diagram (a) is the insulin blank. At 1 mM Pefabloc SC, the formation of the binding is visable as a second peak formation (diagram b). Higher concentrations of the protease inhibitor result in more than one interaction per insulin molecule (diagram c). The special PSC protector eliminates this covalent interaction, even at the highest concentrations (diagram d). Matrix peaks are substracted.



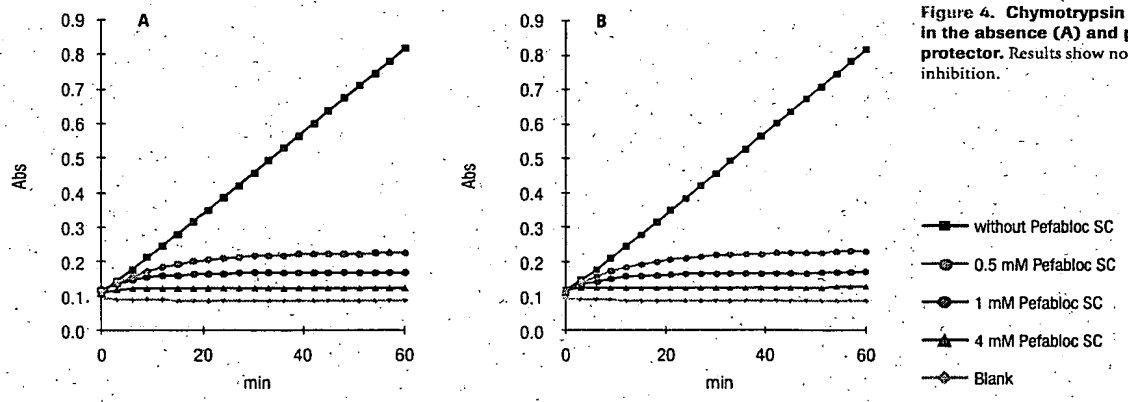
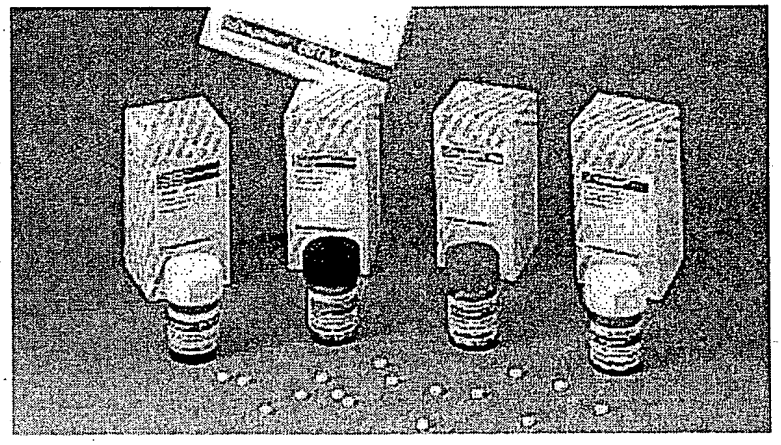


Figure 4. Chymotrypsin inhibition with Pefabloc SC in the absence (A) and presence (B) of the PSC protector. Results show no change in the effectiveness of inhibition.

INTRODUCING NEW
cOmplete
PROTEASE INHIBITOR
COCKTAIL TABLETS

Convenience and Reliability in a cOmplete tablet

cOmplete Protease Inhibitor Cocktail Tablets eliminate the time consuming search for just the right inhibitor. Provided in an easy to use form, simply add one tablet to the aqueous buffer and protect your proteins against a broad range of proteases.



The cOmplete Protease Inhibitor Cocktail Tablets end your time-consuming search for just the right protease inhibitor. These quick dissolving tablets provide the CONVENIENCE and RELIABILITY needed, and end the task of weighing out individual components.

Convenience:

- Mixture of protease inhibitors in one cOmplete tablet can stop a multitude of proteases (Table 1), including serine proteases, cysteine proteases and metalloproteases.
- Use one inhibitor cocktail to work in extracts from almost any tissue or cell, including animals, plants, yeast, bacteria, or fungi.
- Available in two tablet sizes, allowing you to decide the buffer volume.
- Tablets mean never weighing or measuring small quantities anymore.

Reliability:

- Deliver consistent doses of protease inhibition.
- All components provide stable, non-toxic protection in aqueous buffers. EDTA-free tablets do not affect the stability of metal-dependent proteins nor the function of purification techniques (i.e. Poly-His tagged proteins).

cOmplete protection eliminates the worry

Achieve optimal protection with a single tablet. The cOmplete Protease Inhibitor Cocktail Tablets eliminate the questions .... and the doubt.

Table 1: Inhibitions of different proteases by cOmplete Protease Inhibitor Tablets.
One cOmplete tablet was added per 50 ml incubation solution. Proteolytic activity was determined with the Roche Molecular Biochemicals Universal Protease Substrate-casein, resorufin-labeled (Cat. No. 1 080 733). When extractions or single-step isolations are necessary in the acid pH range, simply include Pepstatin along with cOmplete tablets to ensure aspartic (acid) protease inhibition.
All experiments were performed at room temperature.

Source and concentration of protease	Type of protease	% Inhibition immediately after adding cOmplete	% Inhibition 60 min after adding cOmplete
Chymotrypsin, 1.5 µg/ml	Serine	97%	97%
Thermolysin, 0.8 µg/ml	Metallo	99%	100%
Papain, 1 mg/ml	Cysteine	95%	73%
Pronase, 1.5 µg/ml	Mixture	88%	99%
Pancreatic extract, 1.5 µg/ml	Mixture	87%	99%
Trypsin, 0.002 µg/ml	Serine	93%	73%

Classes of Protease Inhibitors available from Roche Molecular Biochemicals

General inhibitors for: Serine proteases[a]	Cysteine proteases[b]	Metallo-proteases[c]	Aspartic proteases[d]
Antithrombin III	E-64	EDTA-Na_2	Pepstatin A*
Aprotinin		Phosphoramidon	
3,4-Dichloroisocoumarin		Bestatin (aminopeptidases)	
APMSF		TIMP-2 (matrix metallo-proteinases)	
Pefabloc SC and Pefabloc SC PLUS			
Leupeptin *(inhibits serine and cystine proteases with trypsin-like specificity)*			
PMSF and PMSF PLUS			
cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets*			
cOmplete Protease Inhibitor Cocktail Tablets*			
α_2-Macroglobulin			

Protease-specific inhibitors	for the inhibition of:
Antipain dihydrochloride	Papain, Trypsin (Plasmin)
Calpain Inhibitor I	Calpain I > Calpain II
Calpain Inhibitor II	Calpain II > Calpain I
Chymostatin	Chymotrypsin
Hirudin	Thrombin
TLCK · HCl	Trypsin, other serine and cysteine proteases (*e.g.*, Bromelain, Ficin, Papain)
TPCK	Chymotrypsin, other serine and cysteine proteases (e.g., Bromelain, Ficin, Papain)
Trypsin-Inhibitor (chicken egg white, soybean)	Trypsin

* When extractions or single step isolations are necessary in the acidic pH range, simply include Pepstatin along with cOmplete tablets to ensure aspartic (acid) protease inhibition.
a) Contain serine and histidine in active center
b) Contain cystine (thiol, SH-) in active center
c) Contain metal ions (e.g. Zn^{+2}, Ca^{+2}, Mn^{+2}) in active center
d) Contain aspartic (acidic) group in the active center

Protease Inhibitor Cocktails

Inhibitor	Specificity of inhibitor	Solubility/ Stability	Suggested starting concentration	Notes
cOmplete Protease Inhibitor Cocktail Tablets (1 tablet used in 50 ml) 1 697 498 20 tablets 1 836 145 3 x 20 tablets	Mixture of several protease inhibitors with broad inhibitory specificity. Inhibits serine, cysteine, and metalloproteases, as well as calpains. Use for extracts from tissues or cells, including animals, plants, bacteria, yeast, and fungi. Contains both reversible and irreversible proteases.	Soluble in aqueous buffers, or add directly to extraction media. Alternatively, prepare 25x stock solutions in 2 ml water or 100 mM phosphate buffer, pH 7.0. Stock solution is stable for 1-2 weeks at 4 °C or at least 12 weeks at –20 °C. All inhibitors in cOmplete can be removed via dialysis. Use of a membrane with cutoff > 10 kDa is recommended. COmplete can be used in thiol-containing solutions at room temperature.	Dissolve one tablet in 50 ml aqueous buffer (without divalent cations) or water. If very high proteolytic activity is present, use one tablet for 25 ml buffer.	For optimal inhibition of metalloproteases, do not prepare protease inhibitor cocktails with buffers containing divalent cations (e.g. Ca^{2+}, Mg^{2+} or Mn^{2+}).**,*** A solution of one cOmplete tablet in 50 ml water has an absorbance of 0.08 at 280 nm.
cOmplete Mini Protease Inhibitor Cocktail Tablets (1 tablet used in 10 ml) 1 836 153 25 tablets	see specificity for cOmplete tablets above	Soluble in aqueous buffers, or add directly to extraction media. Alternatively, prepare 7x stock solutions in 1.5 ml water or 100 mM phosphate buffer, pH 7.0. Stock solution is stable for 1-2 weeks at 4 °C or at least 12 weeks at –20 °C. All inhibitors in cOmplete can be removed via dialysis. Use of a membrane with cutoff > 10 kDa is recommended. COmplete can be used in thiol-containing solutions at room temperature.	Dissolve one tablet in 10 ml aqueous buffer or water. If very high proteolytic activity is present, use one tablet for 7 ml buffer.	see notes for cOmplete tablets above
cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets (1 tablet used in 50 ml) 1 873 580 20 tablets	Mixture of several protease inhibitors that inhibit a broad spectrum of serine and cysteine proteases. Use for extracts from tissue or cells including animals, plants, bacteria, yeast, and fungi. Contains both reversible and irreversible proteases. EDTA-free tablets will not affect the stability or function of metal-dependent proteins.	Soluble in aqueous buffers, or add directly to extraction media. Alternatively, prepare 25x stock solutions in 2 ml water or 100 mM phosphate buffer, pH 7.0. Stock solution is stable for 1-2 weeks at 4 °C or at least 12 weeks at –20 °C. All inhibitors in cOmplete can be removed via dialysis. Use of a membrane with cutoff > 10 kDa is recommended. COmplete can be used in thiol-containing solutions at room temperature.	Dissolve one tablet in 50 ml aqueous buffer or water. If very high proteolytic activity is present, use one tablet for 25 ml buffer.	Does not contain EDTA; thus metal dependent proteins and IMAC isolation techniques (e.g., for Poly-His-tagged proteins) are not affected.**
cOmplete Mini, EDTA-free Protease Inhibitor Cocktail Tablets (1 tablet used in 10 ml) 1 836 170 25 tablets	see specificity for cOmplete, EDTA-free tablets above	Soluble in aqueous buffers, or add directly to extraction media. Alternatively, prepare 7x stock solutions in 1.5 ml water or 100 mM phosphate buffer, pH 7.0. Stock solution is stable for 1-2 weeks at 4 °C or at least 12 weeks at –20 °C. All inhibitors in cOmplete can be removed via dialysis. Use of a membrane with cutoff > 10 kDa is recommended. COmplete can be used in thiol-containing solutions at room temperature.	Dissolve one tablet in 10 ml aqueous buffer or water. If very high proteolytic activity is present, use one tablet for 7 ml buffer.	see notes for cOmplete, EDTA-free tablets above

** Aspartic (acid) proteases exhibit pronounced activity only at low pH. If extraction or single isolation steps must be performed at low pH, simply add Pepstatin to ensure aspartic protease inhibition.
*** If IMAC (immobilized metal chelate affinity chromatography) is to be performed (e.g., for isolating Poly-His-tagged recombinant proteins), remove EDTA via dialysis. As an alternative, use the Complete EDTA-free tablets, available separately.

Individual Protease Inhibitors

Inhibitor	Specificity of inhibitor	Solubility/ Stability*	Suggested starting concentration**	Notes
Antipain-dihydrochloride (Papain Inhibitor) 1 004 646 10 mg 1 004 697 Bulk	Inhibits papain and trypsin. Plasmin is inhibited to a small extent.	Soluble in: H_2O, methanol, DMSO*** to 20 mg/ml. Sparingly soluble in: ethanol, propanol, butanol. Insoluble in: benzene, chloroform ($CHCl_3$), hexane, petroleum and ethyl ethers. Dilute solutions should be stored frozen in aliquots at –20°C. Stable approx. 1 month.	50 µg/ml (74 µM) (1 U of papain is inhibited to 49% by 0.9 µg of antipain.)	Molecular Weight: 677.63 Antipain is more specific for papain and trypsin than is leupeptin. The inibitory potency of antipain is 100-fold higher than that of elastatinal [Ref. 1,2 (p. 683), 3, 4, 5].
Antithrombin III (Heparin Co-factor) from human plasma 602 434 10 Inh. U 410 624 Bulk	Antithrombin III (AT III) inhibits all serine proteases of the blood coagulation system, including thrombin, plasmin, kallikrein, the protease factors IXa, Xa, XIa, and XIIa. It also inhibits trypsin and chymothrypsin. Does not inhibit cysteine proteases, aspartic proteases, and metalloproteases.	Soluble in H_2O (10 mg/ml) Stable in solution for 1 week at +4°C and pH = 7.0–9.0	1 Inh. U/ml Unit definition: One inhibitor unit AT III inactivates 1 U of thrombin (25°C, pH 8.1) in the presence of heparin.	Molecular Weight: 65,000 AT III forms an irreversible 1:1 complex with serine proteases. Once formed, the (AT - thrombin) complex does not dissociate, even during electrophoreses in the presence of denaturing and reducing agents. AT III from Roche is heparin free. The rate of complex formation is accelerated by typically 0.2 units of heparin per unit AT III (Ref. 6, 7).
APMSF (4-Amidino-phenyl) methane-sulfonyl fluoride 917 575 10 mg 973 386 Bulk	Specific and irreversible inhibitor of serine proteases (e.g., trypsin, thrombin, factor Xa, plasmin). Unlike PMSF, APMSF does not inhibit chymotrypsin or acetylcholinesterase.	Can be dissolved in water, soluble to 20 mg/ml. Solution can be stored frozen at –20°C in aliquots. Stability is pH dependent: $t_{1/2}$: pH 6, 20 min; pH 7, 6 min; pH 8, 1 msec.	0.01–0.04 mg/ml (10–20 µM)	Molecular Weight: 216.2 Inhibitory action corresponds to that of DFP, but APMSF is not nearly as toxic (Ref. 34).
Aprotinin 236 624 10 mg 981 532 50 mg 1583 794 100 mg 236 632 Bulk	Serine protease inhibitor. Does not act on thrombin or Factor X. Inhibits plasmin, kallikrein, trypsin, chymotrypsin with high activity.	Freely soluble in H_2O (10 mg/ml) or aqueous buffer solution (e.g., Tris, 0.1 M, pH 8.0). A solution adjusted to pH 7–8 is stable for approx. 1 week at +4°C. Aliquots stored at –20°C are stable approx. 6 months.	0.06–2.0 µg/ml (0.01–0.3 µM)	Molecular Weight: 6,512 Avoid repeated freeze-thaws and exposure to strong alkali solutions. Aprotinin is inactive at pH >12.8 (Ref. 10).
Bestatin 874 515 10 mg 1359 070 50 mg 858 960 Bulk	Primarily, if not exclusively, an inhibitor of amino peptidases and other exopeptidases, including aminopeptidases found in wheat germ and reticulocyte lysate *in vitro* translation systems (e.g., amino-peptidase B, leucine aminopeptidase, tripeptide aminopeptidase, and aminopeptidases on the surface of mammalian cells). It does not inhibit carboxypeptidases.	Soluble to 20 mg/ml in 1 M HCl, 5 mg/ml in methanol, and 1 mg/ml in 0.15 M NaCl. Do not store in HCl. We recommend a stock solution of 2–5 mg/ml in methanol. Solutions are stable for 6 months if stored in aliquots at –20°C.	40 µg/ml (130 µM)	Molecular Weight: 308.4 Bestatin has been found to have antitumor properties and enhances not only blastogenesis and lymphocytes *in vitro*, but also establishes a delayed-type hypersensitivity *in vivo* (Ref. 1, 11–14).
Calpain Inhibitor I (N-Acetyl-Leu-Leu-norleucinal) 1 086 090 25 mg 1 087 177 Bulk	Inhibitor of calpains. Calpains are calcium-dependent neutral cysteine proteases. Inhibits activity of Calpain I: ID_{50} for 0.02 U platelet Calpain I: 0.05 µmol/l. Some inhibitory activity against Calpain II. Inhibits papain to a lesser extent.	Soluble in DMF, ethanol, and methanol to 10 mg/ml. For a stock solution, we recommend dissolving 1 mg of the inhibitor in 100 µl DMF, methanol or ethanol. Before use, dilute with water or phosphate buffer (0.1 M, pH 7.5) to desired concentration. Solutions in DMF, ethanol, or methanol are stable for 2–3 days at +4°C and approx. 4 weeks at –20°C. We recommended making solutions up fresh before use.	17 µg/ml	Molecular Weight: 383.5 Not soluble in water (Ref. 15–18).
Calpain Inhibitor II (N-Acetyl-Leu-Leu-methioninal) 1 086 103 25 mg 1 087 185 Bulk	Inhibits activity of Calpain II. Inhibits Calpain I (ID_{50} = 0.12 µmol/l) and papain to a lesser extent.	(See Calpain Inhibitor I, above).	7 µg/ml	Molecular Weight: 401.6 Not soluble in water (Ref. 15, 16, 17, 19).
Chymostatin 1 004 638 10 mg 1 004 689 Bulk	Specific inhibitor of α-; β-; γ-; δ-chymotrypsin.	Soluble in: glacial acetic acid; DMSO*** to 20 mg/ml. Sparingly soluble in: water, methanol, ethanol. Insoluble in: ethyl acetate, petroleum and ethyl ethers, hexane, chloroform ($CHCl_3$). Dilute solutions should be stored frozen in aliquots at –20°C. Stable approx. 1 month.	6–60 µg/ml (10–100 µM) Unit definition: One unit chymotrypsin is inhibited to 49% by 1.8 µg of chymostatin.	Molecular Weight: 607.71 [Ref. 1, 2 (p. 686), 20]

* Unless otherwise stated, make solutions of inhibitors fresh daily.
** Recommended as a starting concentration. Suitable concentrations must be determined empirically for each new system.
*** CAUTION: DMSO (Dimethyl sulfoxide) will permeate the skin, carrying solubilized protease inhibitors. Always wear appropriate protection for eyes, skin, etc.

Individual Protease Inhibitors

Inhibitor	Specificity of inhibitor	Solubility/ Stability*	Suggested starting concentration**	Notes
3,4-Dichloroisocoumarin 973 840 10 mg 917 184 Bulk	Inhibits a large number of serine proteases such as elastase, cathepsin G, and endoproteinase Glu-C (Staph. V-8 protease).	May be dissolved in DMF and stored in aliquots at –20°C.	1–43 µg/ml (5–200 µM)	Molecular Weight: 215.0 Does not inhibit the thiol protease papain, the metalloprotease leucine aminopeptidase or β-lactamase. More sensitive to hydrolysis than APMSF (Ref. 21).
E-64 (N-(N-(L-3-Trans-carboxirane-2-carbonyl)-L-leucyl)-agmatine) 1 585 673 5 mg 874 523 10 mg 1 585 681 25 mg 858 951 Bulk	Inhibits papain and other cysteine proteases like cathepsin B and L.	Soluble to 20 mg/ml in a 1:1 (v/v) mixture of ethanol and water. Solutions are stable for 1 month if stored in aliquots at –20°C.	0.5–10 µg/ml (1.4–28.0 µM)	Moleacular Weight: 357.4 Stable between pH 2–10. Unstable in strong alkali and strong mineral acids (Ref. 23–25).
EDTA-Na_2 808 261 250 g 808 270 500 g 808 288 1 kg 808 245 Bulk	Inhibits metalloproteases.	Soluble in water to 0.5 M at pH 8–9. Stable at +4°C for at least 6 months.	0.2–0.5 mg/ml (0.5–1.3 mM)	Molecular Weight: 372.24 The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approximately 8.0 by the addition of NaOH (Ref. 22).
EGTA 1 093 053 50 g 1 092 979 Bulk	Specifically inhibits Ca^{++}-dependent proteases.	Stock solution: 200 mg/ml in 1 N NaOH. Stable at +4°C for at least 6 months.	0.2–0.5 mg/ml (0.5–1.3 mM)	Molecular Weight: 380.35. Hardly soluble in water and all organic solvents. Slightly soluble in DMF and DMSO.
Hirudin from *Hirudo medicinalis* (European leeches) 1 110 276 2000 ATU (2 mg)	Specifically inhibits thrombin.	Soluble in 50% ethanol, water, and commonly used buffers. The lyophilizate is stable at room temperature for approx. 2 years. Solutions can be stored at –20°C for at least 6 months.	150–200 ATU/ml plasma Unit definition: One anti-thrombin unit (ATU) neutralizes one NIH unit of thrombin (fibrinogen assay) at 37°C.	
Leupeptin 1 017 101 5 mg 1 017 128 25 mg 1 034 626 50 mg 1 529 048 100 mg 528 595 Bulk	Inhibits serine and cysteine proteases such as trypsin, papain, plasmin, and cathepsin B.	Highly soluble in water (1 mg/ml). Stable for at least 1 week at +4°C and 6 months frozen in aliquots at –20°C.	0.5 µg/ml (1 µM)	Molecular Weight: $C_{20}H_{38}N_6O_4 \times {}^1/_2\ H_2SO_4$: 475.6 $C_{20}H_{38}N_6O_4 \times {}^1/_2\ H_2SO_4 \times H_2O$: 493.6
α_2-Macroglobulin 602 442 25 Inh. U 582 573 Bulk	A general endoproteinase inhibitor. Inhibits most endoproteinases, but does not inhibit endoproteinases that are highly specific for one or a limited number of sequences (e.g., tissue kallikrein, urokinase, coagulation factor XIIa, and endoproteinase Lys-C).	Soluble in water. Stable at least 1 week at room temperature or 3 weeks at +4°C. Can also be frozen in aliquots at –20°C, where it remains stable at least 6 months. Sensitive to acidic pH, denatured below pH 4.0. Ammonia methylamine and hydroxylamine (above pH 7.0) cause irreversible conversion to the inactive form.	Unit definition: One inhibitor unit inhibits 9.1 µg of trypsin.	Molecular Weight: 725,000 Do not use α_2-Macroglobulin in presence of DTT. DTT, even at 1 mM, causes reversible dissociation into inactive subunits. α_2-Macroglobulin acts by physically entrapping the endoproteinases, usually in a 1:1 ratio (Ref. 27).
Pefabloc SC 4-(2-Aminoethyl)-benzenesulfonyl-flouride, hydrochloride (AEBSF) 1 429 868 100 mg 1 585 916 500 mg 1 429 876 1 g 1 427 393 Bulk	Irreversibly inhibits serine proteases, including trypsin, chymotrypsin, plasmin, plasma kallikrein, and thrombin.	Soluble up to 100 mg/ml in aqueous buffers and water. Stable in solution for 1–2 months if stored in aliquots at –20°C. Only slight hydrolysis occurs under weakly basic conditions (pH 8.0–9.0).	0.1–1.0 mg/ml (0.4–4 mM)	Molecular Weight: 239.5 A safe, stable, and water soluble alternative to PMSF and DFP (Ref. 29–32).
Pefabloc SC PLUS 1 873 601 Set I (100 mg Pefabloc SC) 1 873 628 Set II (1 g Pefabloc SC)	Specificity of the protease inhibitor remains unchanged. See Pefabloc® SC.	Solubility and stability of the protease inhibitor remains unchanged. See Pefabloc SC.	0.1–1.0 mg/ml (0.4–4.0 mM)	Sets contain of Pefabloc SC and a special protector (PSC-protector). The set eliminates interaction between Pefabloc SC and sample proteins.
Pepstatin 253 286 2 mg 1 359 053 10 mg 1 524 488 50 mg 253 294 Bulk	Inhibits aspartic (acid) proteases such as pepsin, renin, cathepsin D, chymosin, and many microbial acid proteases.	Soluble in methanol to approx. 1 mg/ml. Also soluble to 1 mg/ml in ethanol if allowed to sit overnight, and to 300 µg/ml in 6 N acetic acid. Stable at least 1 week at +4°C, or 1 month if stored in aliquots at –20°C.	0.7 µg/ml (1 µM)	Molecular Weight: 685.9 Insoluble in water (Ref. 33).

* Unless otherwise stated, make solutions of inhibitors fresh daily.
** Recommended as a starting concentration. Suitable concentrations must be determined empirically for each new system.
*** CAUTION: DMSO (Dimethyl sulfoxide) will permeate the skin, carrying solubilized protease inhibitors. Always wear appropriate protection for eyes, skin, etc.

Individual Protease Inhibitors

Inhibitor	Specificity of Inhibitor	Solubility/ Stability	Suggested starting concentration**	Notes
Phosphoramidon 874 531 5 mg 858 986 Bulk	Specifically inhibits thermolysin, collagenase, and metalloendoproteinases from various microorganisms (*Bacillus subtilis, Streptomyces griseus* and *Pseudomonas aeruginosa*).	Salts of phosphoramidon are soluble to 20 mg/ml in water. Also soluble in methanol and DMSO.*** Recommended stock solution 1–20 mg/ml. Stable in solution for 1 month if stored in aliquots at –20°C.	4–330 µg/ml (7–570 µM)	Molecular Weight: 579.6 (Ref. 1, 2, 11, 36)
PMSF (Phenylmethylsulfonyl fluoride) 236 608 1 g 837 091 10 g 1 359 061 25 g 236 616 Bulk	Inhibits serine proteases (chymotrypsin, trypsin, and thrombin). Also inhibits cysteine proteases such as papain (reversible by DTT treatment).	Soluble to >10 mg/ml in isopropanol, ethanol, methanol, and 1,2-propanediol. Unstable in aqueous solution. In 100% isopropanol, stable at least 9 months at +25°C.	17–170 µg/ml (0.1–1 mM)	Molecular Weight: 174.2 Add fresh PMSF at every isolation/purification step (from stock solution). Does not inhibit metalloproteases, most thiol proteases, and aspartic proteases (Ref. 35).
PMSF PLUS 1 873 636 1 set (contains 1 g of PMSF)	Specificity of the protease inhibitor remains unchanged. See PMSF.	Solubility and stability of the protease inhibitor remains unchanged. See PMSF.	17–680 µg/ml (0.1–4.0 mM)	Set consists of PMSF and a special protector (PMSF-protector®). The set eliminates covalent interaction between PMSF and sample proteins.
TIMP-2 (Tissue Inhibitor of Metalloproteinase 2) from Human Melanoma Cells 1 782 924 10 µg (500 µl)	Inhibits matrix metalloproteinase activity in enzymatic assays and *in vitro* malignant invasion assays.	Supplied in a solution of 20 mM Tris-HCl, pH 7.5, 50 mM NaCl containing 0.02 µg protein per µl. TIMP-2 is stable until the expiry date given on the label if stored at + 20 C. TIMP-2 can be kept at 4 °C for 1 week without significant loss of activity. Repeated freezing and thawing should be avoided.	Specific Activity: 1 µg of TIMP-2 inhibits 0.6 mU Gelatinase 72 kD or Gelatinase 92 kD by 50% in 1 hour at 37 °C.	The protein appears as a major band at 21 kD upon SDS-PAGE. Inactivated by SH-containing compounds (e.g. DTT or DTE).
TLCK · HCl (L-1-Chloro-3-[4-tosylamido]-7-amino-2-heptanone-HCl) 874 485 100 mg 874 493 250 mg 858 943 Bulk	Irreversibly and specifically inhibits trypsin. Also inhibits many other serine and cysteine proteases such as bromelain, ficin, and papain.	Salts of TLCK are soluble to 20 mg/ml in water. We recommend a stock solution of 1 mg/ml in either dilute (1 mM) HCl or buffer, pH ≤ 6; to ensure stability (see "notes" column).	37–50 µg/ml (100–135 µM)	Molecular Weight: 369.3 Stable at +25°C pH ≤ 6.0. Rapidly decomposes at pH >7.5. For example, at pH 9.0, +25°C, TLCK's half-life is only 5 minutes. Chymotrypsin is not inhibited (Ref. 37).
TPCK (L-1-Chloro-3-[4-tosylamido]-4-phenyl-2-butanone) 874 507 1 g 858 935 Bulk	Irreversibly inhibits chymotrypsin. Also inhibits many other serine and cysteine proteases such as bromelain, ficin, and papain.	Soluble to 20 mg/ml in ethanol. Recommended stock solution: 3 mg/ml.	70–100 µg/ml (200–284 µM)	Molecular Weight: 351.9 Trypsin is not inhibed (Ref. 38, 39).
Trypsin Inhibitors from chicken egg white 109 878 1 g 154 440 Bulk **from soybean** 109 886 50 mg 109 894 500 mg 041 963 Bulk	Inhibits trypsin. Soybean trypsin inhibitor also inhibits factor Xa, plasmin, and plasma kallikrein. Neither inhibit metallo, cysteine, and aspartic proteases or tissue kallikrein.	Both are soluble in water. Recommended stock solution: 1 mg/ml. Store frozen in aliquots at –20°C. Stable at least 6 months.	10–100 µg/ml	Molecular Weight: (egg white) 28,000 (soybean) 20,100 Egg white inhibitor is stable at acid pH and labile at alkaline pH. Soybean inhibitor is sensitive to heat, high pH, and protein-precipating solutions.

Protease Inhibitor Set

In certain cases, irregular types of protease activity are encountered. Determining which protease inhibitor to use can be difficult and expensive – unless you use our Protease Inhibitor Set. Consisting of ten different inhibitors, the set provides an easy, economical way to screen for the correct inhibitor.

Protease Inhibitor Set Cat. No. 1 206 893

Inhibitors included in the set	Specificity of Inhibition	Quantity Supplied
Antipain-dihydrochloride	Papain, Trypsin, Cathepsin A and B	3 mg
Aprotinin	Trypsin, Plasmin, Chymotrypsin, Kallikrein	0.5 mg
Bestatin	Aminopeptidases	0.5 mg
Chymostatin	α-, β-, γ-, δ-Chymotrypsin	1 mg
E-64	Cysteine Proteases	3 mg
EDTA-Na$_2$	Metalloproteases	10 mg
Leupeptin	Serine and Cysteine Proteases such as Plasmin, Trypsin, Papain, Cathepsin B	0.5 mg
Pefabloc SC	Serine Proteases	20 mg
Pepstatin	Aspartic Proteases	0.5 mg
Phosphoramidon	Metalloproteinases, specifically Thermolysin	5 mg

Verify protease inhibition

Roche Molecular Biochemicals Universal Protease Substrate offers a fast and highly sensitive way to determine the effectiveness of protease inhibition. Proteases will digest the casein substrate and release resorufin-labeled peptides. By measuring the absorbance of these peptides, you can detect nanogram quantities of proteolytic activity in less than one hour.

Universal Protease Substrat (casein, resorufin-labeled) Cat. No. 1 080 733 – 15 mg, 1 734 334 – 40 mg

c∅mplete inhibition: *the choice is yours*

When deciding on the type of protection to use against unwanted protease activity, the choice is obvious: you want c∅mplete protection! And choosing the appropriate c∅mplete tablet is just as simple. Available in two sizes (regular or mini) and with or without EDTA, our c∅mplete Protease Inhibitor Cocktail Tablets will meet all your needs. The choice is yours!

Application	c∅mplete tablets	c∅mplete, EDTA-free	c∅mplete, Mini	c∅mplete Mini, EDTA-free
Inhibition during initial extraction steps (volumes > 50 ml).	++	++	+	+
Inhibition during subsequent purification protocols (volumes < 50 ml)	+	+	++	++
Inhibition during subsequent purification steps require free divalent cations for further processing.[2]	0	++	0	++
Samples containing high metalloproteolytic activity	++	0	++	0

++ Product of choice
\+ Can also be used
0 Not recommended

1 preparation of stock solutions recommended
2 important for example, with metal chelate chromatography
Poly-His tagged proteins, or protein samples used for signal transduction research

Table 2: Choosing the correct c∅mplet tablet.

Enjoy c∅mplete success!

Whatever your application, the versatility of our c∅mplete Protease Inhibitor Cocktail Tablets are sure to provide the protection needed. Try the c∅mplete Protease Inhibitor Cocktail Tablets today, and see how simple success can be. Your laboratory just isn't complete without it.

Some examples of cells, tissues and organisms of which protease activity has been successfully inhibited with c∅mplete tablets – as reported in scientific literature:

- Brain tissue from rats
- CEM (acute lymphoblastic leukemia)
- Colorectal and duodenal adenomas
- Cos 7 cells
- E.Coli
- Epithelialkidney cells
- HEK 293 cells
- Hela cells
- HL 60
- HMEC (mammary epithelial cells)
- HUVE (human umbilical venous endothelial cells)
- Jurkat cells
- Keratinocytes (mammalian)
- M1 (murine leukaemic cells)
- Mammary gland (mouse)
- MCF7 cells
- MMAC1-mutated glioblastoma cell line U87MG
- Primary lung cancer cells
- Pulmonary arterial smooth muscle cells from rats
- Retina (mammalian)
- RMS (embryonal rhabdomyosarcoma cell line; RD)
- Saccharomyces cerevisiae
- Sf9 cells
- T 24 bladder carcinoma cells
- WEHI 3b D (murine leukaemic cells)

This list of cells is continually updated as we receive new reports. Please visit **http://bi chem.r che.com** on the Internet for a more recent overview.

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Protease Inhibitor Cocktails

Product	Cat. No.	Pack Size
cOmplete Protease Inhibitor Cocktail Tablets	1 697 498 1 836 145	20 tablets 3 x 20 tablets
cOmplete Mini Protease Inhibitor Cocktail Tablets	1 836 153	25 tablets
cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets	1 873 580	20 tablets
cOmplete Mini, EDTA-free Protease Inhibitor Cocktail Tablets	1 836 170	25 tablets

Individual Protease Inhibitors

Product	Cat. No.	Pack Size
Antipain dihydrochloride	1 004 646	10 mg
Antithrombin III	602 434	10 IU
APMSF	917 575	10 mg
Aprotinin	236 624 981 532 1 583 794	10 mg 50 mg 100 mg
Bestatin	874 515 1 359 070	10 mg 50 mg
Calpain Inhibitor I	1 086 090	25 mg
Calpain Inhibitor II	1 086 103	25 mg
Chymostatin	1 004 638	10 mg
3,4-Dichloroisocoumarin	973 840	10 mg
E-64	1 585 673 874 523 1 585 681	5 mg 10 mg 25 mg
EDTA-Na_2	808 261 808 270 808 288	250 g 500 g 1 kg
EGTA	1 093 053	50 g
Hirudin	1 110 276	2000 ATU (2 mg)
Leupeptin	1 017 101 1 017 128 1 034 626 1 529 048	5 mg 25 mg 50 mg 100 mg

Product	Cat. No.	Pack Size
α_2-Macroglobulin	602 442	25 IU
Pefabloc SC	1 429 868 1 585 916 1 429 876	100 mg 500 mg 1 g
Pefabloc SC PLUS	1 873 601 1 873 628	Set I (contains 100 mg Pefabloc SC and 5 ml PSC protector solution) Set II (contains 1 g Pefabloc SC and 2 x 25 ml PSC protector solution)
Pepstatin	253 286 1 359 053 1 524 488	2 mg 10 mg 50 mg
Phosphoramidon	874 531	5 mg
PMSF	236 608 837 091 1 359 061	1 g 10 g 25 g
PMSF PLUS	1 873 636	1 Set (contains 1 g PMSF and 35 ml protector solution)
Protease Inhibitor Set	1 206 893	1 set
TIMP-2	1 782 924	10 µg (500 µl)
TLCK · HCl	874 485 874 493	100 mg 250 mg
TPCK	874 507	1 g
Trypsin Inhibitor (chicken egg white)	109 878	1 g
Trypsin Inhibitor (soybean)	109 886 109 894	50 mg 500 mg
Universal Protease Substrate (Casein, resorufin-labeled)	1 080 733 1 734 334	15 mg 40 mg

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